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<p>(21) International Application Number: PCT/US88/01213 (22) International Filing Date: 20 April 1988 (20.04.88) (31) Priority Application Number: 042,178 (32) Priority Date: 24 April 1987 (24.04.87) (33) Priority Country: US (71) Applicant: BIOGEN N.V. [NL/NL]; Pietermaai 15, Willemstad, Curaçao (AN). (71)(72) Applicant and Inventor: FISHER, Richard, A. [US/US]; 9-1 Auburn Court, Brookline, MA 02146 (US). (72) Inventors: GILBERT, Walter ; 107 Upland Road, Cambridge, MA 02140 (US). SATO, Vicki, L. ; 43 Larch Road, Cambridge, MA 02138 (US). RAMACHANDRAN, Kuzhalmannam, L. ; 24 Otis Street, Natick, MA 01760 (US).</p>		<p>(74) Agents: HALEY, James, F., Jr. et al.; Fish & Neave, 875 Third Avenue, New York, NY 10022-6250 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent). Published <i>With international search report.</i></p>
<p>(54) Title: IMMUNOTHERAPEUTIC METHODS AND COMPOSITIONS</p>		
<p>(57) Abstract</p> <p>This invention relates to immunotherapeutic methods and compositions. More particularly, this invention relates to methods for eliciting specific antibody responses in immunodeficient patients using immunogens comprising antigen/T-cell independent carrier conjugates. According to a preferred embodiment, this invention relates to immunogens comprising peptides from the <i>env</i> region of the HIV genome coupled to T-cell independent carriers, which are useful in preventing or treating acquired immune deficiency syndrome and AIDS related complex.</p>		

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IMMUNOTHERAPEUTIC METHODS
AND COMPOSITIONS

TECHNICAL FIELD OF INVENTION

5 This invention relates to immunotherapeutic methods and compositions. More particularly, this invention relates to methods for eliciting specific antibody responses in immunodeficient patients using immunogens comprising antigen/T-cell independent
10 carrier conjugates. According to a preferred embodiment, this invention relates to immunogens comprising peptides from the env region of the HIV genome coupled to T-cell independent carriers, which are useful in preventing or treating acquired immune deficiency
15 syndrome and AIDS related complex.

BACKGROUND ART

 In immunocompetent individuals, T-4 lymphocytes, also known as helper or inducer cells, interact with other specialized cell types of the
20 immune system to confer immunity to or defense against infection. More specifically, T-4 lymphocytes stimulate production of growth factors which are critical to the functioning of the immune system. For example, they activate macrophages ("killer cells") to attack
25 infected or otherwise abnormal host cells, and induce monocytes ("scavenger cells") to encompass and destroy invading microbes. They also induce maturation of B

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lymphocytes into cells which produce defensive antibodies to specific antigens. The induction of antibody formation by T-dependent antigens requires interaction with both T lymphocytes and B lymphocytes, with the T lymphocytes somehow regulating the proliferation and differentiation of B lymphocytes into antibody secreting cells by mechanisms not yet fully understood. B lymphocyte differentiation and antibody formation may occur, however, without T lymphocyte induction, in response to T-independent antigens or, possibly, supplies of exogenous growth factors.

This complex immune defense system may be partially or totally disrupted as a result of a given therapeutic regimen. For example, chemotherapy and radiation therapies used to treat malignant diseases, or immunosuppressants administered to prevent rejection of transplanted organs, often cause selective destruction of circulating T-4 lymphocytes or contribute to other T lymphocyte abnormalities in the patient undergoing treatment. Such side effects of these treatments may lead to complete immunosuppression and attendant susceptibility of the patient to a wide range of opportunistic infections. Even when immunosuppression is not complete, in those patients having depleted T lymphocyte populations, exposure to a given antigen alone may not be sufficient to elicit the formation of antibodies.

Similar immunosuppression is seen in patients suffering from acquired immune deficiency syndrome ("AIDS"). The human immunodeficiency virus ("HIV") retrovirus is thought to be the etiological agent responsible for AIDS infection and its precursor, AIDS related complex ("ARCS") [M. G. Sarngadharan et al., "Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) From Patients With AIDS and Pre-AIDS",

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Science, 224, pp. 497-508 (1984)].* Between 85 and 100% of the AIDS/ARCS patient population test sero-positive for HIV [G. N. Shaw et al., "Molecular Characterization of Human T-Cell Leukemia (Lympho-
5 tropic) Virus Type III In The Acquired Immune Deficiency Syndrome", Science, 226, pp. 1165-70 (1984)]. Upon infection of a host, the primary targets of the HIV virus, T-4 lymphocytes, are rendered non-functional. In addition to T-4 lymphocytes, the HIV
10 virus are also been shown to infect central nervous system cells, macrophages and B lymphocytes [J. M. Ismach, "AIDS: Can The Nation Cope", Medical World News (August 25, 1985)].

To date, the need exists for the development
15 of effective immunotherapeutic agents and methods for the treatment of immunodeficiencies caused by T lymphocyte depletion or abnormalities.

DISCLOSURE OF THE INVENTION

The present invention solves the problems
20 referred to above by providing immunotherapeutic methods and compositions for use in the treatment of immunodeficient patients. The methods and compositions of this invention are characterized by immunogens which comprise antigens specific for a target
25 infection coupled to T-cell independent carriers. These immunogenic conjugates, which are advantageously used in patients who are immunosuppressed due to T lymphocyte depletion or abnormalities, elicit the formation of antibodies specific for target

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* In this application, human immunodeficiency virus ("HIV"), the generic term adopted by the human retrovirus subcommittee of the International Committee on Taxonomy of Viruses to refer to independent iso-
35 lates from AIDS patients, including human T-cell lymphotropic virus type III ("HTLV-III"), lymphadenopathy-associated virus ("LAV") and AIDS-associated retrovirus ("ARV") will be used.

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infections. Accordingly, the methods and compositions of this invention provide therapeutic or prophylactic immunogenic responses in immunocompromised patients who lack functioning T lymphocyte systems.

5 The antigen/T-cell independent carrier conjugates of this invention may be advantageously used in therapeutic compositions or vaccines which elicit antibodies reactive with target infections. According to one preferred embodiment of this inven-
10 tion, immunogens comprising peptides from the env region of the HIV genome coupled to T-cell independent carriers may be used to elicit antibodies reactive with the native env protein of the HIV virus. Such immunogens are useful in preventing or treating
15 acquired immune deficiency syndrome and AIDS related complex.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the amino acid sequences of each of peptides 1-2, 4-6, 31, 64 and 78 used in
20 one embodiment of the antigen/T-cell independent carrier conjugates of this invention, as well as that of the region between amino acid 600 and amino acid 750 of the HIV env gene. In this figure, the amino acids are represented by single letter codes
25 as follows:

Phe: F	Leu: L	Ile: I	Met: M
Val: V	Ser: S	Pro: P	Thr: T
Ala: A	Tyr: Y	His: H	Gln: Q
Asn: N	Lys: K	Asp: D	Glu: E
30 Cys: C	Trp: W	Arg: R	Gly: G

DETAILED DESCRIPTION OF THE INVENTION

According to this invention, antigens specific for a target infection are coupled to one or more T-cell independent carriers, for example
35 carriers such as Ficoll, lipopolysaccharide ("LPS"), dextran sulfate or Staphylococcus aureus Cowan strain,

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before use. The antigens may also be coupled to any other conventional polymeric T-independent carrier to which peptides may be conjugated. These antigens are coupled to the carrier according to a procedure such as that set forth in Example 2 of this application. Alternatively, the antigens may be coupled to the carrier in various conventional ways using, for example, methods using glutaraldehyde [M. Reichlin, "Use Of Glutaraldehyde As A Coupling Agent For Proteins And Peptides", Methods In Enzymology, 70, pp. 159-65 (1980), N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide [T. L. Goodfriend et al., "Antibodies To Bradykinin And Angiotension: A Use Of Carbodiimides In Immunology", Science, 144, pp. 1344-46 (1964)] or a mixture of N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide and a succinylated carrier [M. H. Klapper et al., "Acylation With Dicarboxylic Acid Anhydrides", Methods In Enzymology, 25, pp. 531-36 (1972)].

Antigens which may be coupled to T-independent carriers to form immunogenic conjugates according to this invention include peptides involved in the pathogenesis of the HIV virus. Such peptides include peptides from the env region of the HIV genome, for example, peptides characterized by an amino acid sequence derived substantially from the region between about amino acid 600 and amino acid 750 of the HIV env gene or the D-retro forms of those peptides -- those produced by synthesis with D amino acids in the opposite orientation, beginning with the carboxy terminal amino acid of the L form.

Additionally, antigens which may be coupled to T-independent carriers to form the immunogenic conjugates of this invention include those which are specific for opportunistic infections afflicting immunosuppressed or immunocompromised patients. Such antigens include, for example, antigens directed against bacterial pneumonia, pneumocystis pneumonia,

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hepatitis, varicella virus, cytomegalovirus and
Epstein-Barr virus. These antigens may be prepared
according to any conventional method including, for
example, chemical synthesis or recombinant DNA tech-
nology.

After preparing the antigen and coupling
it to the T-cell independent carrier, the antigen/
T-cell independent carrier conjugate is employed in
the methods and compositions of this invention in a
conventional manner. For example, the coupled anti-
gen, alone or in combination with other coupled anti-
gens of this invention, may be mixed with one or a
combination of well-recognized adjuvants and addi-
tives, preferably by first dissolving the coupled
antigen, for example, in PBS with 0.1% SDS. In other
embodiments of this invention, coupled antigens may
be linked to hydrophobic groups to build the adjuvant
into the composition. In addition, the coupled anti-
gen may be administered to a patient in combination
with lymphokines, such as B cell growth factors,
which are known to stimulate the activity of B
lymphocytes. Of course, it should be understood
that other well-known methods of preparing therapeutic
compositions may be employed using the antigen/T-cell
independent carrier conjugates of this invention.

The above-prepared compositions are then
employed in a conventional manner for the treatment
of opportunistic infections in immunodeficient
patients. Such methods of treatment and their dosage
levels and requirements are well-recognized in the
art and may be chosen by those of skill in the art
from available methods and techniques. For example,
the conjugates of this invention may be combined
with a pharmaceutically acceptable adjuvant for
administration to patient in a pharmaceutically
acceptable manner and in an amount effective to elicit
antibodies specific for the target infection and to

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lessen the severity of that infection. The dosage and treatment regimens will depend upon factors such as the patient's health status, the severity and course of infection and the judgment of the treating physician.

Alternatively, the antigen/T-cell independent carrier conjugates of this invention are useful in vaccines and methods for protecting immunodeficient humans against opportunistic infections for at least some period of time. The conjugates may be employed in these vaccines and methods either alone or together with other conjugates of this invention in a manner consistent with the conventional utilization of antigens in vaccines. For example, the antigen/T-cell independent carrier conjugates of this invention may be combined with pharmaceutically acceptable adjuvants conventionally employed in vaccines and administered in immunologically effective amounts to protect immunodeficient patients for some time against opportunistic infections. Additionally, the immunogenic conjugates may be combined with B lymphocyte-stimulating factors for administration as a vaccine.

Both the compositions and vaccines of this invention may be administered to patients via conventional modes of administration. The frequency of administration will depend upon factors such as the particular composition or vaccine employed and the condition of the patient. The need for subsequent treatments with these compositions or boosters of these vaccines will depend upon the results of the initial treatment or vaccination. The compositions, vaccines and methods of this invention may be used to treat human as well as other mammalian patients.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are

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not to be construed as limiting this invention in any manner.

In these examples, we coupled peptides involved in the pathogenesis of HIV infection to T-cell independent carriers to form the conjugates of this invention and tested the conjugates in several conventional assays to demonstrate their ability to elicit anti-HIV antibody responses in immunodeficient hosts. It should be understood that the present invention also relates to conjugates comprising the D-retro form of each of these illustrative peptides.

Example 1 - Preparation Of Peptides
Involved In The Pathogenesis
of HIV Infection

Referring now to Figure 1, we synthesized peptides 1-2, 4-6, 31, 64 and 78, corresponding to segments of the env gene of the HIV genome. In Figure 1, the amino acid numbering corresponds to that set forth for the env gene in L. Ratner et al., "Complete Nucleotide Sequence Of The AIDS Virus, HTLV-III", Nature, 313, pp. 277-84 (1985).

We synthesized the peptides using an improved version of the solid phase method described by R. B. Merrifield, "Solid Phase Peptide Synthesis. I. The Synthesis Of A Tetrapeptide", J. Am. Chem. Soc., 83, pp. 2149-54 (1963), using an Applied Biosystems Model 430A peptide synthesizer and reagents and procedures as supplied by the producer. In this improved method, we deblocked and cleaved the protected peptides from the resin with liquid HF containing 10% anisole, in a variation of the method described by S. Sakakibara et al., "Use Of Anhydrous Hydrogen Fluoride In Peptide Synthesis. I. Behavior of Various Protective Groups In Anhydrous Hydrogen Fluoride", Bull. Chem. Soc. Jap., 40, pp. 2164-68 (1967).

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We first purified the peptides cleaved from the resin by partition chromatography on a Sephadex LH20 column using n-Butanol/water (6/100) as eluent. The eluate was further purified by semi-preparative high pressure reversed phase chromatography on an Altex Ultrasphere-ODS column, by elution with a 0.1% TFA acetonitril gradient. After we hydrolyzed the eluate with 6N HCl for 18 hours, we carried out amino acid analysis on a Beckman amino acid analyzer to confirm the amino acid sequences of the peptides produced.

Example 2 - Coupling Of An HIV-Peptide
To A Carboxymethyl Ficoll
Carrier

We prepared carboxymethyl Ficoll according to the procedure described in J. K. Inman, "Thymus-Independent Antigens: The Preparation Of Covalent, Hapten-Ficoll Conjugates", J. Immunol., 114, pp. 704-09 (1975). We then dissolved 3 mg of the carboxymethyl Ficoll in 100 μ l of 2mM hydrochloric acid. Subsequently, we added a solution of N-hydroxy-succinimide (1 mg) in 20 μ l of 2mM hydrochloric acid. To this mixture, we added a solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1 mg) in 10 μ l water. We mixed the reaction mixture well and reacted it for between about 25 and 30 minutes to preactivate the carboxymethyl Ficoll.

We then coupled the carboxymethyl Ficoll to a peptide involved in the pathogenesis of HIV infection -- for, example, peptides 4, 5 and 6, as depicted in Figure 1. We did this by adding a solution of 3.0 mg of the peptide in 150 μ l of 0.3M sodium bicarbonate and adjusting the total volume to 400 μ l with 0.3M sodium bicarbonate. We allowed the coupling reaction to proceed for between about 4 and 4 1/2 hours at room temperature.

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We then loaded the reaction mixture onto a Sephadex G100 column (10 inches x 0.325 inches) which had been pre-equilibrated with 1X PBS (pH 7.2). Subsequently, we eluted the column with 1X PBS to obtain the peptide-Ficoll conjugate. We collected a total of 20 x 30 drop fractions and read the absorbance at 214 nm (" A_{214} ") of each fraction. We pooled and lyophilized the void volume fractions showing A_{214} . The lyophilized solid was then taken up in 1 ml of sterile water and a 50 μ l aliquot was removed. To that aliquot, we then added 50 nM of Norleucine, lyophilized to dryness and then hydrolyzed with constant boiling hydrochloric acid for between about 18 and 24 hours for amino acid analysis. The amount of peptide coupled to carboxymethyl Ficoll ranged between about 10 to 20 peptide molecules per molecule of Ficoll.

Example 3 - Inoculation Of Test Animals

We dissolved each of the coupled peptides prepared above in sterile PBS, to a final concentration of 0.5 μ g/ μ l coupled peptide in PBS. Subsequently, we inoculated 7-8 week old athymic nu/nu CD1 strain female mice (Charles River Breeding Laboratories) or immunocompetent BALB/C (Charles River Laboratories) or BALB/CJ mice (Jackson Laboratory, Bar Harbor, Maine) by intraperitoneal injection of 0.2 ml of the coupled peptide. All mice were prebled to establish an average baseline for each response to be measured. The dosage of the coupled peptide varied with the nature of the immunogen and no adjuvants were administered. Tail bleeds were taken on days 4 and 6, with serum being stored as individual samples at -20°C until being pooled at the time of assay.

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Example 4 - ELISA With Anti-Peptide/T-Cell
Independent Carrier Conjugate
Sera Against HIV env Peptide
Coated Plates

5 In this assay, we determined that antiserum
raised in an animal by each of the peptide/T-cell
independent carrier conjugates of this invention
binds to its respective peptide. Accordingly, the
coupled peptides of this invention are immunogenic
10 and elicit a response in test animals.

To carry out the assay, we coated two
96-well microtiter plates (Nunc Immuno I) with 50 μ l
per well of a mixture of 50 μ g/ml uncoupled peptide
in PBS. (20mM phosphate, 150mM NaCl, pH = 7.2) and
15 incubated the plates overnight at 4°C. The third
and fourth plates, which served as controls for,
respectively, the first and second plates, were
treated identically to those plates but were not
pre-coated with the peptide. We then inverted the
20 plates to empty all wells and washed the plates
3 times with PBS/0.05% Tween-20. The plates were
blotted dry by gentle tapping over paper towels.
After blotting the plates, we added 150 μ l of a 5%
fetal calf serum in PBS solution ("FCS/PBS") to each
25 well and incubated the plates for 1 hour at room
temperature. We then washed and blotted the plates
as before.

We then assayed serum samples pooled from
each group of 3 mice on the pre-coated plates prepared
30 as described above and on two control plates. In
the first pre-coated plate, we assayed the antibody
response to the immunogen peptide at an initial dilu-
tion of 1:10, followed by serial 2-fold dilutions in
5% FCS/PBS.

35 After a 2 hour incubation period at room
temperature, we washed the plates and blotted them
dry as described above. We then added 50 μ l of a
1:2000 dilution of goat anti-mouse-IgM horsera-

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dish peroxidase (μ specific) ("HRP") in 5% FCS/PBS to each well and incubated the plates at room temperature for 1 hour. We then washed the plates with PBS/0.05% Tween-20. We added 500 μ l of 42mM 3, 3', 5, 5'-tetramethylbenzidine in dimethylsulfoxide ("TMB/DMSO") and 7.35 μ l of 30% hydrogen peroxide ("H₂O₂") to 50 ml of 0.1M sodium acetate-citric acid buffer (pH = 4.92). Subsequently, we added 50 μ l of this solution to the wells using a 12 channel multiple pipet. We stopped the enzyme reactions with 50 μ l of 2M H₂SO₄ when the less dilute samples reached an absorbance of 0.2 O.D. at 650 nm. We then analyzed the plates spectrophotometrically at 450 nm using an automated microplate reader (Dynatech MR600) and observed that antiserum against each of the peptide/T-cell independent carrier conjugates prepared in Example 2 binds to its respective peptide.

Example 5 - ELISA With Anti-Peptide/T-Cell Independent Carrier Conjugate Sera Against Virus-Coated Plates

In this assay, we demonstrated that antisera raised against the peptide/T-cell independent carrier conjugates of this invention binds to HIV virus-coated plates.

We added 100 μ l of carbonate buffer (pH = 9.6) containing 5% bovine serum albumin to each well of 96 well microtiter plates coated with authentic HIV virus (a gift of Dr. Robert Gallo)* and incubated

* It is our understanding that the plates used were in fact made, or at least could have been made, by coating 96 well microtiter plates (Nunc Immuno I) with 100 μ l of a mixture of 5 μ g/ml authentic HIV virus in carbonate buffer (pH = 9.6), incubating the plates overnight at 4°C, inverting the plates to empty all wells, washing the plates 3 times with deionized water and then blotting them.

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the plates at room temperature. Virus-coated micro-titer plates are also available from Electronucleonics, Fairfield, New Jersey. Subsequently, we rinsed the plates 3 times with deionized water.

5 After blotting the plates, we added 100 μ l of saline- PO_4 (PBS) containing 5% normal goat serum (filter for 0.22 μ m Nalgene filter) to each well and incubated the plates for 2 hours at room temperature. We next added 100 μ l of test serum or control serum
10 to each well and incubated the plates for 2 1/2 hours at room temperature. The test serum comprised pooled samples of antisera raised against the peptide 4/Ficoll conjugate of this invention at dilutions of 1:10 or 1:25. We then washed the plates 3 times with PBS
15 containing 0.05% Tween-20 and blotted them.

We next added 100 μ l of a 1:1000 dilution of 1% normal goat serum and goat anti-mouse-IgM HRP (μ specific) in 0.05% PBS-Tween 20 to each well and incubated the plates for 1 hour at room temperature.
20 We had titrated the anti-mouse IgM HRP before use to assure a proper final concentration of indicator antibody. At the end of the hour incubation period, we rinsed the plates 2 times with 0.05% PBS-Tween-20 and once with plain PBS.

25 We then added 100 μ l of a solution of 0.005% H_2O_2 and 0.05% orthophenylene diamine ("OPD")* in Sorenson's phosphate citrate buffer (pH = 5) and allowed reaction for 20 minutes at room temperature in the dark.

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* This is a potential carcinogen which should be detoxified before disposal using a solution of:

35 50 g K_2CrO_7
25 ml 10N H_2SO_4
145 ml H_2O

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We stopped the reaction by adding 50 μ l of 4N H_2SO_4 to each well. The plates were read by visual inspection or using a microplate reader at 450 nm.

Each plate had a series of "blank" control wells containing no test serum or anti-mouse IgM-HRP conjugate and to which one of the following had been added:

- saline- PO_4 (PBS) containing 20% normal goat serum
- 10 -- PBS-Tween-20 (0.05%)
- Sorenson's phosphate-citrate buffer (pH = 5) containing 0.005% H_2O_2 and 0.05% OPD.

In addition, each plate had a series of "background" control wells containing no test serum and to which one of the following had been added:

- saline- PO_4 (PBS) containing 20% normal goat serum
- 20 -- PBS-Tween-20 (0.05%) containing 1% normal goat serum and goat - anti-mouse-IgM HRP at a dilution of 1:4000
- Sorenson's phosphate-citrate buffer (pH = 5) containing 0.005% H_2O_2 and 0.05% OPD.
- 25

Each test plate also had a negative and positive control serum.

Analysis of the plates revealed that anti-serum raised against coupled peptide 4 bound to the HIV virus. Although antisera against coupled peptides 5 and 6 were not tested in this assay, we believe that these antisera may exhibit the binding activity demonstrated by antiserum raised against coupled peptide 4. This demonstrates that methods and compositions of antigen presentation according to this invention elicited an antibody that recognizes HIV.

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While we have hereinbefore presented a number of embodiments of this invention, it is apparent that our basic construction can be altered to provide other embodiments which utilize the process of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the claims appended hereto rather than the specific embodiments which have been presented hereinbefore by way of example.

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We Claim:

1. A pharmaceutically acceptable composition for treating or preventing a target infection in immunosuppressed patients comprising an immunologically effective amount of an immunogen which comprises an antigen specific for that infection coupled with a T-cell independent carrier.
2. The composition according to claim 1, wherein the target infection is an HIV infection and the antigen comprises a peptide involved in the pathogenesis of HIV virus selected from the group consisting of peptides characterized by an amino acid sequence derived substantially from the region between about amino acid 600 and amino acid 750 of the HIV env gene.
3. The composition according to claim 2, wherein the peptide is selected from the group consisting of peptides consisting substantially of amino acid sequences of the formulae: PWNASWSNKSLEQIWN, LLELDKWASLWNWF, EQIWNNTWMEWD, LPIPRGPDRPEGIEEGGERDRDR and D-retro forms thereof.
4. The composition according to claim 1, wherein the target infection is an HIV infection and the antigen comprises a peptide involved in the pathogenesis of HIV virus selected from the group consisting of peptides consisting substantially of amino acid sequences of the formulae: RIKQIINMWQEVGKAMYAPPISQI, VKIEPLGVAPTKAKRRVVQREKRA, NSSSGRMIMEKGGEIKNCS, SVEINCTRPNNNTRKSI and D-retro forms thereof.
5. The composition according to claim 1, wherein the T-cell independent carrier is selected

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from the group consisting of Ficoll, lipopolysaccharide; dextran sulfate and Staphylococcus aureus Cowan strain.

5 6. A method for treating an immunosuppressed patient comprising the step of administering to said patient an immunologically effective amount of a composition according to any one of claims 1 to 5.

10 7. A method for treating HIV infection in a patient comprising the step of administering to said patient an immunologically effective amount of a composition according to any one of claims 2 to 5.

15 8. A method for treating a patient to protect them for some time against HIV infection comprising the step of treating said patient in a pharmaceutically acceptable manner with a composition according to any one of claims 2 to 5.

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FIGURE 1

AA 600- : GIWGCSGKLICTTAVPWNAS
750 of WSNKSLEQIWNMTWMEWDR
HIV env EINNYTSLIHSLIEESQNQQ
EKNEQELLELDKWASLWNWF
NITNWLWYIKLFIMIVGGLV
GLRIVFAVLSVVNRVRQGYS
PLSFQTHLPIPRGPDRPEGI
EEEGGERDRD

Peptide 1 : PWNASWSNKSLEQIWN
(AA 616-632
of HIV env)

Peptide 2 : LLELDKWASLWNWF
(AA 667-680
of HIV env)

Peptide 4 : LPIPRGPDRPEGIEEGGERDRDR
(AA 728-751
of HIV env)

Peptide 5 : RIKQIINMWQEVGKAMYAPPISGQI
(AA 426-450
of HIV env)

Peptide 6 : VKIEPLGVAPTKAKRRVVQREKRA
(AA 496-519
of HIV env)

Peptide 31 : NSSSGRMIMEKGEIKNCS
(AA 148-165
of HIV env)

Peptide 64 : EQIWNMTWMEWD
(AA 627-639
of HIV env)

Peptide 78 : SVEINCTRPNNNTRKSI
(AA 298-314
of HIV env)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/01213

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC (4) C07K 7/08, 7/10; A61K 37/02, 37/20		
US Cl. 530/327, 326, 325, 324 ; 514/12, 13, 14; 424/88		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
US	530/327, 326, 325, 324; 514/12, 13, 14; 424/88	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
CHEMICAL ABSTRACTS AND BIOLOGICAL ABSTRACTS ONLINE COMPUTER SEARCH		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X Y	US, A, 4,185,090 (McINTIRE) 22 January 1980. See column 2 in particular.	1,5 1-8
X Y	US, A, 4,629,783 (COSAND) 16 December 1986. See claims 3 and 6 in particular.	1-4, 6-8 3-8
X Y	PCT WO 86/02383 (INSTITUT PASTEUR) 24 April 1986. See claims 8 and 15 in particular.	1-4, 6-8 3-8
X Y	SCIENCE, (Washington, D.C., USA) volume 231, issued March 1986. (KENNEDY et al.), "Antiserum to a synthetic peptide recognizes the HTLV-III envelope glycoprotein". Pages 1556-1559. See page 1556 in particular.	1-4, 6-8 3-8
<p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
24 MAY 1988	18 JUL 1988	
International Searching Authority	Signature of Authorized Officer	
ISA/US	CHRISTINA CHAN	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

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Y

BIO/TECHNOLOGY (New York, USA) volume
3, issued October 1985, (CHANG et al)
"Detection of Antibodies to human
T-cell lymphotropic virus-III (HTLV-III)
with an immunoassay employing a
recombinant Escherichia coli-derived
viral antigenic peptide", Pages 905-
909. See Fig. 1 in particular.

3-4,
6-8
1-8

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.